

Figure 1: Northern blot analysis of the expression of the cysteine proteinase (CcCP1) gene in different tissues of *Coffea arabica*.

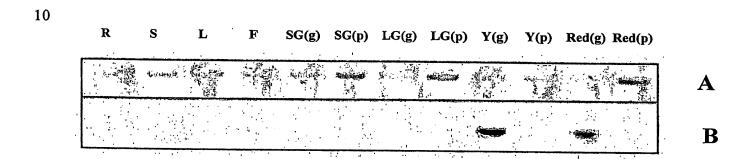
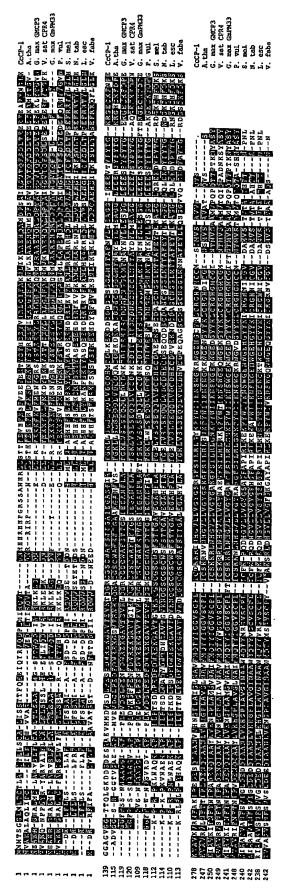
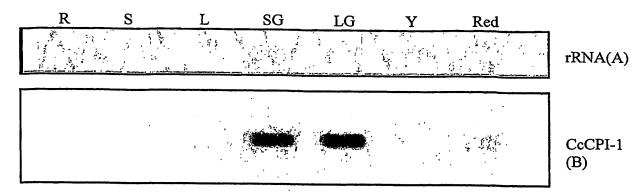


Figure 2: Northern blot analysis of the expression of the Cysteine proteinase CcCP-1 gene in different tissues of Coffea arabica.



protein encoded by CcCP-1 cDNA with other full-length cysteine proteinases available sequence of the Figure 2A: Alignment of the full database.



5 **Figure 3**: Northern blot analysis of the expression of the cysteine proteinase inhibitor (CcCPI-1) gene in different tissues of *Coffea arabica*.

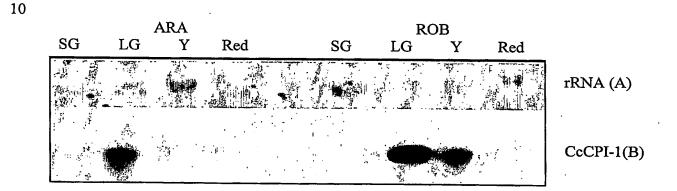


Figure 4: Northern blot analysis of the expression of the cysteine proteinase inhibitor gene (CcCPI-1) at different cherry development stages for *Coffea arabica* (ARA) and *Coffea canephora* (ROB).

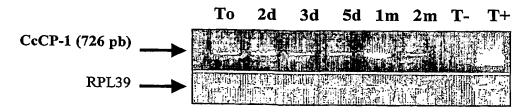


Figure 5. RT-PCR analysis of the expression of CcCP-1 during Coffea arabica grain germination.

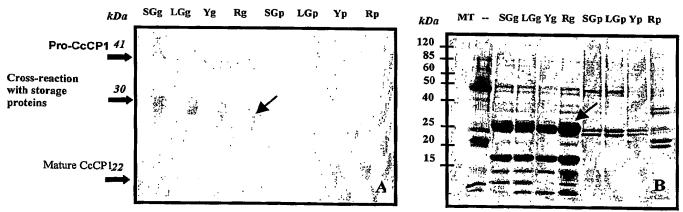
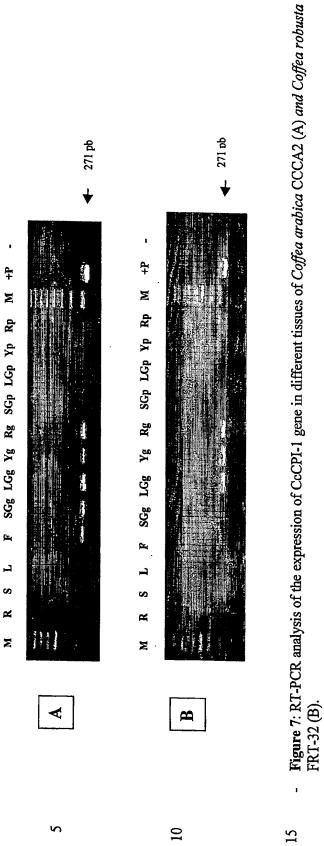


Figure 6: Western-blot analysis of the expression of CcCP1 protein (A).

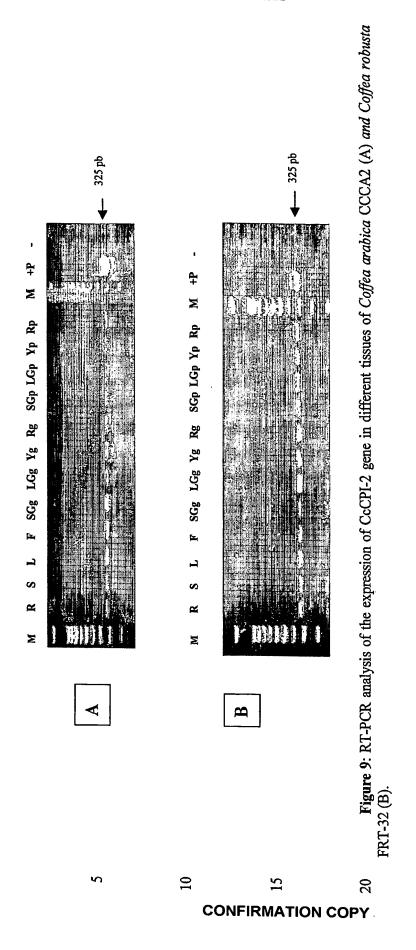
CPI-1 M. dosmest Sunflower R. obtusif A. thalian	CPI-1 M. dosmest Sunflower R. obtusif A. thalian	CPI-1 M. dosmest Sunflower R. obtusif A. thalian
DVKSNKF NVKTNK NVKTDT GSANSL NIRTNR	A E K Q V V A A Q S Q V V S A A B T Q V V V A A B T C Q V V V A A T K E Q V V V A A Q K Q V V V A A Q K Q V V V A A Q K Q V V V A A A Q K Q V V V A A A Q K Q V V V A A A Q K Q V V V A A A Q K Q V V V A A A Q K Q V V V A A A Q K Q V V V A A A Q K Q V V V A A A Q K Q V V V A A A Q K Q V V V A A A Q K Q V V V A A A Q K Q V V V A A A Q K Q V V V A A Q K Q V V V A A Q K Q V V V A A Q K Q V V V A A Q K Q V V V A A Q K Q V V V A A Q K Q V V V A A Q K Q V V V A A Q K Q V V V A A Q K Q V V V A A Q K Q V V A A Q V V A Q V V A Q V V A A Q V V	A T K . P K V D K C O G S T S
3 A R E K I E G G R R E I E G G G R R E G G G G G G G G G G G G G	- E I I F T S V V E A E K Q V V V A C A E K Q V V V A C A E K Q V V V A C A E C K Q V V V A C A E C C A C C A C C A C A C A C A C	N F S P S P G F F K F A P G G F F K F A P G G F T F A P G G F T F A P G G F T P V V
I L I F E I L A L E S T T L Q V N A L G R K V G A R E K I E D V K S N K E I V A S N G Y G G M V G G R K E I E N V K T N K E E I E L V A T A I P G G R T K V K N V K T D T E I E L V A T A I P G G R T K V K N V K T D T E I E L V A T I G G I K Q V E G S A N S L E I E L V V T P S A A N P F R K S V V L G G K S G V P N I R T N R E	L R K K N N E S G A P I I F T S V V E A E K Q V V A A R G T Q K M D G G G E I D E E V V E A Q S Q V V S A Q S Q V V S A Q S Q V V S A Q S Q V V S A Q S Q V V S A Q S Q V V S A Q S Q V V S A Q S Q V V S A Q S Q V V S A Q S Q V V A A Q N A M	V P X V Y D A I V V V R P W V H T K P R O L L L N F S P S P A T K . V H R L F D S E V V V K P W L R S K Q L L D N F A P H G P K K M K V F D A E V V V Q S W K H S K K L L G F K F A P V D K K X V Y E A K V W V K P W M N F K Q V Q E F K L L G D Q G S T S T R M F D S V V V I Q P W L H S K Q L L G F T P V V S P V Y
D 1 1 1 D 1 1 1 1 D 1 1 1 1 D 1 1 1 1 D 1 1 1 1 D 1 1 1 1 D 1 1 1 1 D 1 1 1 2	N N F F F F F F F F F F F F F F F F F F	V V R P W L R R V H V V V V V R P W L R R W W V V Q S W K H W W V K P W M N W V K P W M N W V K P W L H L H L H L H L H L H L H L H L H L
S - 1 L A L F L L A L C L V A T L L L L L L L L L L L L L L L L L L	M M M M M M M M M M M M M M M M M M M	K V Y D; A I V R L F D; A E V K V F D; A E V K V Y E A V V R F D S V
	Y N K Y Y N K Y N K Y N K Y N K Y N K Y N K Y N K Y N K Y N K Y Y N N Y Y N N O O O	R N - G V P P Y K G G K M F D G - G K K M F Q P N G S T F R D G S T F F R F F F F F F F F F F F F F F F F
S S S L L T L L L L L L L L L L L L L L	G R F S V E E E G S F S V E E E E G S F S V E E E E E E E E E E E E E E E E E E	X L K L K A T Y Y L K A T Y Y L K V S A V Y Y L K L E A T Y I T L E A T Y L R L E A T Y L R L E V T T K L E V T T Y L R L E V T T K L E V T T T K L E V T T T K L E V T T T K L E V T T T K L E V T T T T T T T T T T T T T T T T T T
1 M M M K V I I M M M K V I I M M M K V I I I M M I I K V V V V V V V V V V V V V V V V V	52 V Q E L 40 V Q E L 39 I Q Q L 19 V E S L 48 I Q Q L	93 G I K Y 79 G T K Y 53 G T M Y 99 G L K Y

Figure 6A: Optimal alignment of the complete protein encoded by CcCPI-1 cDNA with other homologous full-length cysteine proteinases available in the NCBI.



CcCPI-2 R. obtusifolius D. caryophyllus M. esculenta	CcCP1-2 R. obtusifolius D. caryophyllus M. esculenta	CcCPI-2 R. obtusifolius D. caryophyllus M. esculenta
2 N A L L E E N N N A L L E E E E E N N A L L E E	 X Y W W X W W	
D Y N K K O H N K K O H N K K K C K K K K K K C K K K K K K K K	K	
E N S L E I E S L A K F A V D D Y N K K Q N A L L E E A N S L E V E S L A K F A V E D H N K K Q N A M L E A N S L E I D N L A K F A V D D Y N K K Q N A L E E E A N S V E I D N L A R F A V D D Y N K K Q N A L E E	V A G T I E V F F K L Y E A V K P V F P F	
E I E S L E I E S L E I E I E I E I E I E I E I E I E I E	L T I E V I I T I L E A I I T L E A I I T L E A I I T L E A I I T L E A I I T L E A I I T L E A I I T L E A I I T L E A I I T L E A I I T L E A I I T L E A I I T L E A I I T L E A I I T L E A I I T L E A I I T L E A I I T L E A I I T L E A I I T L E A I T	A . T S D S T A
N R R R R R R R R R R R R R R R R R R R	A G T V Y Y Y A A G T M Y Y Y A G T I Y Y Y Y A G T M Y Y Y	A A G D T S A . L G D Q G S T S V G D A S A I G V A P S D S T A
S E S K G K K D S V E G G K K E V E E E	E 0 V V V V V V V V V V V V V V V V V V	Q E F K L Q D F K Y Y Y Q D F K Y Y Y
A K V G G 1 A T V G G G 1 A T V G G G 1 A T L G G G 1 A T L G G G 1 A T L G G G 1 A T L G G G 1 A T L G G G 1 A T L G G G 1 A T L G G G 1 A T L G G G 1 A T L G G G 1 A T L G G G 1 A T L G G G G G G G G G G G G G G G G G G	Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q	M N F K Q V N F K E V V N F K E V V N F K E V
	40 40 41 7 7 7	80 81 81 80 80

Figure 8: Optimal alignment of the complete protein encoded by CcCPI-2 cDNA with other homologous full-length cysteine proteinases available in the NCBI.



CcCPI-3 Citrus x paradisi A. deliciosa A. thaliana	CcCPI-3 Citrus x paradisi A. deliciosa A. thaliana	CcCPI-3 Citrus x paradisi A. deliciosa A. thaliana	CcCPI-3 Citrus x paradisi A. deliciosa A. thaliana
A L V G G W A L V G G W V A A G G W A R V G G W	E A Q K K G E T S G E T	X X X X X X X X X X X X X X X X X X X	
K D A L K G A L K L V A A A A R R	L E F K T V V E K L Q Y Q S V V R L K F E T V V S	W L K F K K W W E H F K W W M K F R	
A L G G R P A A G D R - V V G G R - P L Y A S -	7 K L E F F D D E L Q Y F F S G L K F F F F F F F F F F F F F F F F F F	V W E K P V W D K P	
I C L F S D V P S A A L G G R P K D A L V G G W P C - F L S V V P L L A G D R - K G A L V G G W L L L L A L S A A V V G G R - K L V A A G G W S L - V V L L L P L Y A S - A A R V G G W	K E A G K Q S K Q A N K Q A N K R S B E	N L Y E A I V W V K P W L K K K F A I V W D K P W W H F I A I I V W D K P W M K	
F L S V V V V V L L L L A L S L - V V V	F A I D E H N K E A G F A V T E Y N K I Q S K F A V S E H N K Q A N F A V S E Y N K R S E	S A V V S V	
T A A I C	E N G K F A I D E H N K E A G G I O V A Q F A V T E Y N K Q S K D V A Q F A V S E H N K Q A N E I G E F A V S E Y N K R S E	K A L D G T - V V K D G P - A A K D G A - A A N D G D G G	
V L L T T I	E V L E N E I Q V V E I Q	1 V J K A L L V V V L V J A A A A A A A A A A A A A A A A A A	я Л
P H L L L L S L I L V F L L L	P K D P P K E K L N S A V T D P P	X X X X X X X X X X X X X X X X X X X	Р . V К N N G R
M M M M M M M M M M M M M M M M M M M	S - K A D K P I E D R P I E S S S P I S N	Q V V A G G Q V V V S G G	S F K P M S F R K V S F E P A
	41 33 32	80 73 72	

Figure 10: Optimal alignment of the complete protein encoded by CcCPI-3 cDNA with other homologous full-length cysteine proteinases available in the NCBI.

CcCPIA Citrus x paradisi A. thaliana	CcCPI-4 Citrus x paradisi A. thaliana	CcCPI-4 Citrus x paradisi A. thaliana
T V N P K D P H V I Q	Y M L A I K T Q D L T Y R L I L V V K D G P Y R L K V A A N D G D	
A G Q K N M V G G G L S S T V P P R S S T V N P K D P R D P P R D P P R D P P R D P P P P	T V V W L N V,E Y G F W W I D D D T Y M L A I K T Q D L T A L K F E S V E K G E T Q V V S G T N Y R L I L V V K D G P G L X F E T V V S G T Q V V S G T N Y R L K V A A N D G D	S N G T Y S L K W Y N H N N K. E H - F K S L T S F K P M V K M K - F R N L . S F E P A N G R F L
T A A I G V I F I S L I S L -	N A K A G T N K Q S K S N K S E S	V R H I S E V W W E K P W W V W D K P W
1 M A T V A A K S A 1 M N Q R F C C L I 1 M T S K V V F L L	46 1 A Q F A V A N Y 46 1 G Q F A V T E Y 45 1 G E F A V S F V	91 - G T H C D V A L 91 - S T K K F E A V 90 G V S K N Y L A I

Figure 11: Optimal alignment of the complete protein encoded by CcCPI-4 cDNA with other homologous full-length cysteine proteinases available in the NCBI.

S

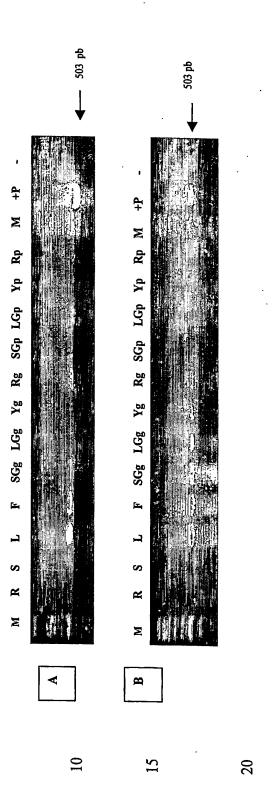


Figure 12: RT-PCR analysis of the expression of CcCPI-4 gene in different tissues of Coffea arabica CCCA2 (Panel A) and of Coffea robusta FRT-32 (Panel B)

PCT/EP2004/006805

 ${\tt 1} \ {\tt gcttacatcttaaatcctgattttatagattcgcctttcgtgaagttcaatcttcgcagtcgctcactaacatttggt}$ 81 agacatacttcgatt ATG AAA ATG GGG AAG GCT TTC CTT TTT GCC GTT GTA TTG GCT GTG ATC MKMGKAFLFAVVL v 144 TTA GTG GCG GCT ATG AGC ATG GAG ATC ACA GAA AGA GAT TTG GCT TCT GAG GAA AGC TTG A A M S M E 204 TGG GAC TTG TAC GAA AGA TGG AGG AGC CAT CAT ACT GTT TCT CGA GAC CTT TCT GAG AAA R R S H 264 CGA AAG CGC TTT AAT GTT TTC AAG GCA AAT GTC CAT CAC ATT CAC AAG GTG AAC CAG AAG F ĸ A N H H 324 GAC AAG CCT TAC AAG CTG AAA CTC AAC AGT TTC GCT GAT ATG ACC AAC CAC GAG TTC AGG K L N S F A M 384 GAA TTC TAC AGT TCT AAG GTG AAA CAT TAC CGG ATG CTC CAC GGC AGT CGT GCT AAT ACT v s ĸ R M H 444 GGA TTT ATG CAT GGG AAG ACT GAA AGT TTG CCA GCC TCC GTT GAT TGG AGA AAG CAA GGA E G K T 8 L 504 GCC GTG ACT GGC GTC AAG AAT CAA GGC AAA TGT GGT AGC TGT TGG GCA TTT TCA ACT GTG G K N Q G K С G S С W 564 GTT GGA GTC GAG GGA ATC AAC AAA ATC AAA ACA GGC CAA TTA GTT TCT CTG TCC GAG CAA I N K I K G O. Ŀ 624 GAA CTT GTT GAC TGT GAA ACG GAC AAT GAA GGA TGC AAC GGA GGA CTC ATG GAA AAT GCA D С E T С N G G M 684 TAC GAG TTT ATT AAG AAA AGT GGG GGA ATA ACA ACT GAG AGG CTA TAT CCC TAC AAG GCA I ĸ ĸ S G G 744 AGA GAT GGC AGC TGT GAT TCG TCA AAG ATG AAT GCC CCT GCT GTG ACT ATT GAT GGG CAT D S S K M 804 GAA ATG GTA CCC GCA AAC GAT GAG AAT GCC TTG ATG AAA GCT GTT GCT AAC CAG CCT GTA 237 v D E N A L М ĸ A A 864 TCA GTA GCT ATA GAT GCG TCT GGC TCT GAC ATG CAA TTT TAT TCA GAG GGT GTA TAC GCT I D Α G S S D M 924 GGA GAC TCG TGT GGC AAT GAG CTT GAT CAT GGC GTG GCG GTC GTC GGC TAC GGG ACT GCT С G N E L D H G v A 984 CTT GAC GGT ACT AAA TAC TGG ATA GTG AAG AAC TCA TGG GGA ACA GGA TGG GGA GAA CAG ĸ W 1044 GGC TAT ATC AGG ATG CAA CGT GGT GTT GAT GCT GCT GAA GGC GGA GTT TGT GGG ATA GCA C 1104 ATG GAG GCC TCC TAT CCA CTT AAA TTG TCC TCC CAC AAT CCA AAA CCA TCC CCA CCT AAG ĸ S A S Y P L L 3 H L 1240 agactacgcgcttcTGaagacttagatcatctctaggcatagatttatgtaatcctgctcctgtgatggtttgaataaac

Figure 14: cDNA sequence and its deduced amino acid sequence of CcCP-4. Lowercase: 5' and 3' non-translated regions; Uppercase: Open reading frame; Bold character: amino acid sequence; *: stop codon

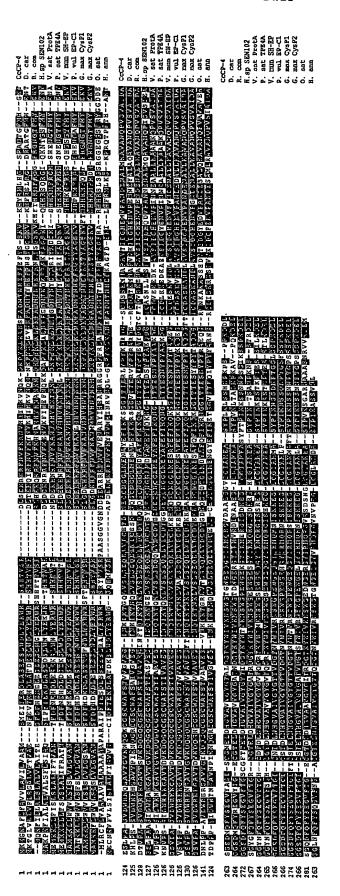


Figure 15: Alignment of the full sequence of the protein encoded by CcCP-4 cDNA with other full-length cysteine proteinases available in the NCBI database 9

	GSCFIACATCTAAATCCTGATTTTTATAGATTCGCCTTTCGTGAAGTTCAATCTTCGCAGTCGCTCACTACATTTGGTAGACATTAGTAGTATTATAGATTATAGAA	KDEL -CCC KDDL -CCC	
1 101	AATGSGGAAGGCTTTOCTTTTGCCGTTGTATTCGCTGTGTGCGGCTATGAGCATGSAGATCACAGAAAGATTTGGGTTCTCAAAGAGATTTGGGTTCTCAAAGAG	KDEL -CCC KDDL -CCC	
1 201	TIGISGAC FIGIACGAA GAIGGA GCCAICAIACIGITIC. CGAGACDITICI GAGAAACGAAACGAAAIGTITAAAAATTGICCAAA	KDEL -CCC KDDL -CCC	
1 301	ACATTCACAASGTSAACCAGAAAGGCTTACAAGUTGAACTCAACATTTCGCTGATATGACTAAGAGTTCASG3AATTCTAGAGTTCTAA	KDEL -CCC KDDL -CCC	
1 401	SGT GARACATTACOSGATGOTCACGCAGTCSTGCTAATACTGGATTTATGCGTAGGGAAGACTGAAAGTTTAGCCAGCC	KDEL -CCC KDDL -CCC	
1 501	GGAGCCGTGACT GGCGTCAAGAAAAAAAAAAAAAAAAAA	KDEL -CcC KDDL -CcC	
74 601	AATTAGTTICICTGTCCGAGCAAGAACITGTIGACITGTAAACGGACAATGPAGGATGCAACGGACCGAGGACCGAAAATGCATACAAGTTTAATTAA	KDEL -CCC KDDL -CCC	
174	AAGFGGGGGAATAACAACTGAGACCTATATCCCTACAAGGCAAGGAFGGCAGDTGTGATTCATCAAAGATGAAFGCCCCTGCTGAGCAGCATTGATGAGAAAAAAAAAA	KDEL -CCC KDDL -CCC	
274 801	JATGAAATGGTACCCGCAAACGATGAGAATGCCTTGAAGGGTGTTGGGAAAGCGAGCCTGTATACAGATGCGTATGGATGCGTCTGGGTCTGCAAT JATGAAATGGTACCCGCAAACGATGAGAATGCCTTGATGATGCTTGCT	KOEL -CCC KODL -CCC	
374 901	TTTATTCAGAGGGTGTATAC <mark>A</mark> CTGGAGAJTU <mark>A</mark> TGTGGCCAAZ 3AGJTTGATCATGSCGTGGCGGTCGTCGGCTA <mark>R</mark> 3GAACTGCCCTTGACJCTAGAAAAA TTTATTCAGAGGGTGTATACGCTGGAGAGTGGGCAAASAGGTTGATCATGGCGTGGGGGCGTCGGGGGAACTAGGGGGGACGGGTACGGGAGTTGAGTAAATA	KDEL -CCC KDDL -CCC	
474	OTGSATACTCANGNACTCATGGGGAACA SGATGGGGGGGAAACAGGGGCTATATCAGGPTACAACGTGGTGT. SATJCTGGGGGGGGGGGGGTTTGTGGGANTA OTGSATAGTGAAGAACTCATGGGGAACAAGAGAGAGAGAGAGGGTTATATCAGGATGTGGGTGTTGATGGTGGGGGGGG	KDEL -CCC KDDL -CCC	
574 1101	SCAATGSAGGCCTCCTATCCACTTAAAFTGTCCTCCCACAATCCAAAACCATCCCCACCTAAGGACGAGCTCTAGATTGATCTTTATATATA	KDEL -CCC KDDL -CCC	
674 1201	CATATATATATATATATATATATITTO i stagattuatigaatittagitacagactacgcctinigaaggettagatcatctotaggcatagnittatg k tatatatatataticagtagattuatteaatittag	KDEL -CCC KDDL -CCC	
774 1289	TAATCCTGCTCCTGTGATGGTTTGAATAAABAATAAGTACCNTNTHAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	KDEL -CCC KDDL -CCC	
Decora	Decoration 'Decoration #1': Shade (with solid black) residues that match KDDL -CcCP4 exactly.		

Figure 16. The full length cDNA sequence CcCP-4 KDDL and the partial cDNA sequence CcCP-4 (KDEL).

- -	MKNGKAFLFAVVLAVILVAAMSMEITERDLASEESLWDLYERMRSHHTVSRDLSEKRKRFNVFKANVHHIHKVNQKDKPY	CCCP-4 KDDI CCCP-4 KDEI	KODI	
81	KLKLNSFADNTNHEFREFYSSKVKHYRMLHGSRANTGFMHGKTESLPASVDWRKQGAVTGVKNQGKCGSCWAFSTVYGVE	CCCP-4	KDDI	
161 17	GINKIKTGOLVSLSEQELVDCETDNEGCNGGLMENAKEFIKKSGGITTERLYPYKARDGSCDSSKHNAPAVTILGHENVP GINKIKTGOLVSLSEQELVUCETINKGCNGGLMENAYEFIKKSGGITTERLYPYKARDGSCDSSKHNAPAVTIDGHENVP	CCCP-4 KDDI	KDDI KDEI	
241 97	ANDENALMKAVANQPVSVAIDASGSDMQFYSBGVYAGDSCGNET,DHGVAVVGYGTALDGTKYWIVKNSWGTGWGEQGYLR ANDENALMKAVANQPVSVAIDASGSDMQFYSBGVY <mark>M</mark> GDSCGNELDHGVAVVGYGTALDGTKYWIVKNSWGTGWGEQGYIR	CCCP-4 KDDI	KDDI KDEI	
321 177	NQRGYDAABGGYCGIAMBASYPLKLSSHNPKPSPPKDDL. NQRGYDAABGGYCGIAMBASYPLKLSSHNPKPSPPKDBI.	CCCP-4 KDDI CCCP-4 KDEI	KODI	
Decoi	Decoration 'Decoration #1': Shade (with solid black) residues that match CCCP-4 KDD, exactly.			

Figure 17. The complete open reading frame of CcCP-4 (KDDL) and the partial open reading frame of CcCP-4 (KDEL).

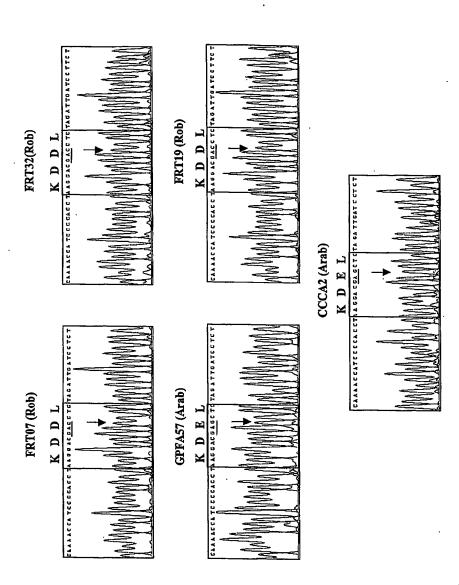


Figure 18. DNA sequence chromatograms for PCR amplified genomic DNA encoding the KDEL/KDDL region of the CcCP-4 gene.

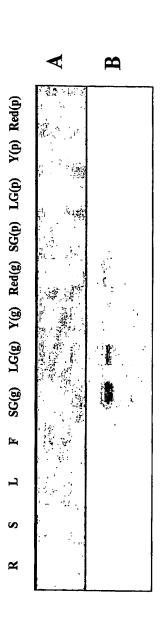


Figure 19. Northern blot analysis of the expression of the Cysteine proteinase CcCP-4 gene in different tissues of Coffea arabica.

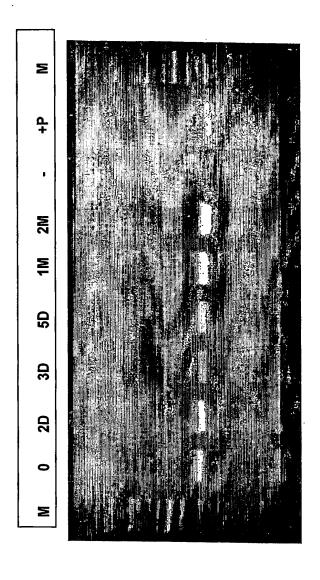


Figure 20. RT-PCR analysis of the expression of CcCP-4 in the whole grain during germination.

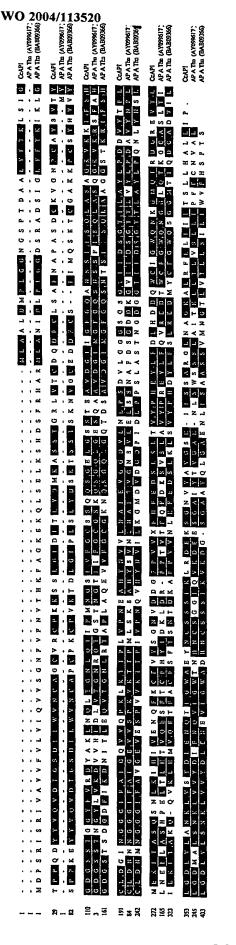
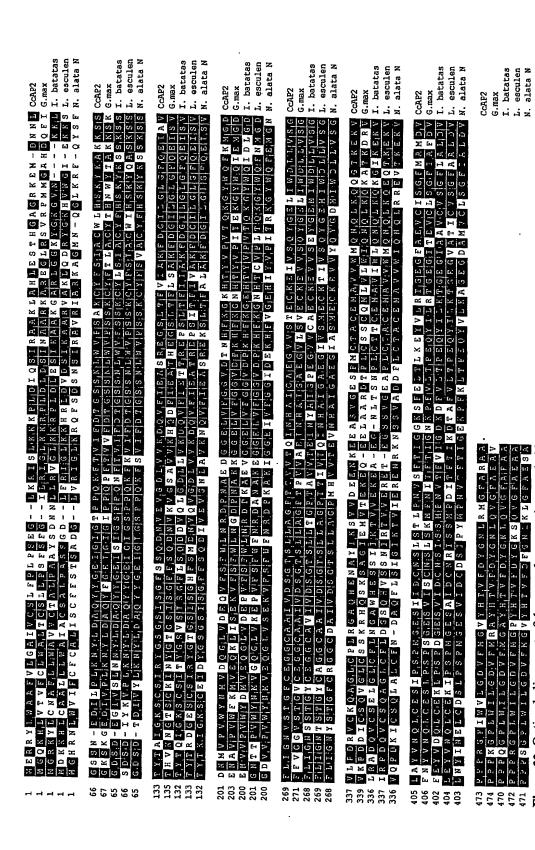


Figure 21: Optimal alignment of the complete protein encoded by CcAP-1 cDNA with other homologous full-length aspartic proteinase sequences available in the NCBI



aspartic proteinase CcAP-2 cDNA with other homologous full-length encoded by compl alignment of the Optimal